BD FastImmune™ γ2a/γ1/CD4/CD3 is designed as the isotype control for the detection of intracellular cytokines and expression of the activation marker CD69 in antigen-activated CD4+ T lymphocytes in whole blood. Applications include studies of T-cell responses to antigens such as cytomegalovirus (CMV),1-5 human immunodeficiency virus (HIV),6,7 herpes viruses,1 and tumor antigens.8

**RESEARCH APPLICATIONS**

**DESCRIPTION**

**Specificity**

Both γ2a (IgG2a) and γ1 (IgG1) react specifically with keyhole limpet hemocyanin (KLH), an antigen not expressed on human cells or human cell lines.

The CD49,10 antibody recognizes an antigen, with a molecular weight of 55-kilodalton (kDa)11 that is present on T-helper/inducer lymphocytes and monocytes.12,13

The CD3 antibody reacts with the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex.14 This complex is composed of at least six proteins that range in molecular weight from 20 to 30 kDa.15 The antigen recognized by CD3 antibodies is noncovalently associated with either α/β or γ/δ TCR (70 to 90 kDa).16

**Antigen distribution**

The CD4 antigen is present on the helper/inducer T-lymphocyte subset, such as CD4+CD4+, that comprises 28% to 58%17 of normal peripheral blood lymphocytes.11,13 It is also present on 80% to 95% of normal thymocytes.11,13 The CD4 antigen is present in low density on the cell surface of monocytes and in the cytoplasm of monocytes and macrophages (CD3−CD4+).18 The CD4 antigen is the receptor for the HIV.19 Some CD4 antibodies, including CD4, inhibit HIV binding to CD4+ cells.20 Subjects infected with HIV were found to exhibit a continuous loss of CD4+ lymphocytes and a relative increase in the CD8 (Leu-2a)+ lymphocyte subset.21-23

The CD3 antigen is present on 61% to 85% of normal peripheral blood lymphocytes.17

**Clones**

γ2a (IgG2a), clone X39, and γ1 (IgG1), clone X40, are both derived from hybridization of Sp2/0-Ag14 mouse myeloma cells with spleen cells from BALB/c mice immunized with KLH.

CD4, clone SK3, is derived from hybridization of NS-1 mouse myeloma cells with spleen cells from BALB/c mice immununized with human peripheral blood T lymphocytes.

CD3, clone SK7, is derived from hybridization of NS-1 mouse myeloma cells with spleen cells from BALB/c mice immunized with human thymocytes.

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**Composition**

γ₂ₐ (clone X39) is composed of mouse IgG₂ₐ heavy chains and kappa light chains.

γ₁ (clone X40), CD4, and CD3 are each composed of mouse IgG₁ heavy chains and kappa light chains.

The BD FastImmune γ₂ₐ/γ₁/CD4/CD3 reagent is supplied as a combination of γ₂ₐ FITC, γ₁ PE, CD4 PerCP-Cy™ 5.5, and CD3 APC in 1.0 mL of phosphate-buffered saline (PBS) containing bovine serum albumin (BSA), beta-lactoglobulin, and 0.1% sodium azide.

**PROCEDURE**

For complete activation and staining protocol and the appropriate application note, visit our website (bdbiosciences.com) or contact your local BD representative.

**Abbreviated Intracellular Staining Procedure**

1. Add 1 mL of 1X BD FACS™ lysis solution (Cat. No. 349202) to 100 µL of activated heparinized whole blood.
2. Incubate 10 minutes at room temperature.
3. Centrifuge at 500 x g for 5 minutes; decant the supernatant.
4. Add 0.5 mL of 1X BD FACS™ Permeabilizing Solution 2 (Cat. No. 340973).
5. Vortex and incubate for 10 minutes at room temperature.
6. Wash by adding PBS containing 0.5% BSA and 0.1% sodium azide (NaN₃), and centrifuge for 5 minutes.
7. Add 20 µL of BD FastImmune γ₂ₐ FITC/γ₁ PE/CD4 PerCP-Cy5.5/CD3 APC.
8. Vortex and incubate for 30 minutes at room temperature in the dark.
9. Repeat wash step; resuspend cells in 1% paraformaldehyde in PBS.

**REPRESENTATIVE DATA**

Performed on SEB-activated whole blood. Laser excitation is at 488 nm and 635 nm.

**Figure 1** Representative data analyzed with a BD FACS™ brand flow cytometer

**HANDLING AND STORAGE**

Store vials at 2°C–8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

**WARNING**

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

**CHARACTERIZATION**

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.
WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

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REFERENCES


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